Remarks

None of the amendments adds new matter.

The amendments correct formal matters. Specifically, the amendments bring the specification into conformity with the formal drawings submitted herewith and correct the address of the ATCC.

Accordingly, Applicants respectfully request that this Amendment be entered.

Respectfully submitted,

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Version with markings to show changes made

The paragraph beginning at page 5, line 2:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the HOIPS I polypeptide having the amino acid sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2) or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host with the American Type Culture Collection ("ATCC"), [12301 Park Lawn Drive, Rockville, Maryland 20852]Patent Depository, 10801 University Boulevard, Manassas, VA, 20110-2209, on December 16, 1996. (ATCC Deposit Number 97825).

The paragraph beginning at page 6, line 8:

[Figure 1 shows]FIGs 1A-1B show the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of HOIPS I. The protein has a leader sequence of about 20 amino acid residues and a deduced molecular weight of about 17.8 kDa. The predicted amino acid sequence of the mature HOIPS I protein is also shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2).

The paragraph beginning at page 6, line 13:

[Figure 2]FIG. 2 shows the regions of similarity between the amino acid sequences of the HOIPS I protein and chicken MD-1 (SEQ ID NO:3). The consensus sequence is shown (SEQ ID NO:17).

The paragraph beginning at page 6, line 16:

[Figure 3]FIG. 3 shows an analysis of the HOIPS I amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown. In the "Antigenic Index - Jameson-Wolf" graph, amino acid residues about 17 to about 29, about 33 to about 39, about 43 to about 52, about 56 to about 67, about 74 to about 83, about 90 to about 94, about 110 to about 120, about 125 to about 139, and about 145 to about 152 in [Figure 1]FIGs 1A-1B correspond to the shown highly antigenic regions of the HOIPS I protein. These highly antigenic fragments in [Figure 1]FIGs 1A-1B correspond to the following fragments, respectively in SEQ ID NO:2: amino acid residues about -4 to about 9, about 13 to about 19, about 23 to about 32, about 36 to about 47, about 54 to about 63, about 70 to about 74, about 90 to about 100, about 105 to about 119, and about 125 to about 132.

The paragraph beginning at page 7, line 2:

The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding a HOIPS I polypeptide having the amino acid sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2), which was determined by sequencing a cloned cDNA. The HOIPS I protein of the present invention shares sequence homology with the chicken MD-1 protein. [(Figure 2)](FIG. 2) (SEQ ID NO:3). The nucleotide sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) was obtained by sequencing the HTOCD71X clone, which was deposited on December 16, 1996 at the American Type Culture Collection, [12301 Park Lawn Drive, Rockville, Maryland 20852]Patent Depository, 10801 University Boulevard, Manassas, VA, 20110-2209. (ATCC accession number 97825) The deposited clone is contained in the pBluescript SK(-) plasmid (Stratagene, LaJolla, CA).

The paragraph beginning at page 8, line 3:

Using the information provided herein, such as the nucleotide sequence in [Figure 1]FIGs 1A-1B, a nucleic acid molecule of the present invention encoding a HOIPS I polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) was discovered in a cDNA library derived from human tonsils tissue. The gene was also identified in cDNA libraries from the following tissues: bone marrow, dendritic cells, fetal and adult brain macrophages, B cells, and lymph nodes. The determined nucleotide sequence of the HOIPS I cDNA of [Figure 1]FIGs 1A-1B (SEQ ID NO:1) contains an open reading frame encoding a protein of 162 amino acid residues and a deduced molecular weight of about 17.8 kDa. The HOIPS I protein shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2) is about 45% identical to, and about 64% similar to, the chicken MD-1 protein [(Figure 2)](FIG. 2) in a 132 amino acid residue overlap.

The paragraph beginning at page 8, line 17:

The present invention also provides the mature form(s) of the HOIPS I protein of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is

ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature HOIPS I polypeptides having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) and as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2). By the mature HOIPS I protein having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) is meant the mature form(s) of the HOIPS I protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature HOIPS I having the amino acid sequence encoded by the cDNA clone contained in the host deposited with the ATCC on December 16, 1996, (ATCC Deposit No. 97825) may or may not differ from the predicted "mature" HOIPS I protein shown in [Figure 1]FIGs 1A-1B (amino acids from about 1 to about 142 in SEQ ID NO:2) depending on the accuracy of the predicted cleavage site based on computer analysis.

The paragraph beginning at page 9, line 19:

In the present case, the predicted amino acid sequence of the complete HOIPS I polypeptides of the present invention were analyzed by a computer program ("PSORT") (K. Nakai and M. Kanehisa, *Genomics 14*:897-911 (1992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated. The analysis by the PSORT program predicted the cleavage sites between amino acids 20 and 21 in [Figure 1]FIGs 1A-1B (SEQ

ID NO:2). Thereafter, the complete amino acid sequences were further analyzed by visual inspection, applying a simple form of the (-1,-3) rule of von Heinje. von Heinje, *supra*. Thus, the leader sequence for the HOIPS I protein is predicted to consist of amino acid residues -20 to -1 in SEQ ID NO:2. However, while the predicted mature HOIPS I protein consists of residues 1-142, the present inventors have identified other possible cleavage sites resulting in mature proteins having the following amino acid residues shown in SEQ ID NO:2: -7-142, -6-142, -5-142, -4-142, -3-142, -2-142, -1-142, 2-142, 3-142, 4-142, 5-142, 6-142, 7-142, 8-142, 9-142, 10-142, 11-142, 12-142, 13-142, 14-142.

The paragraph beginning at page 10, line 26:

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1); DNA molecules comprising the coding sequence for the mature HOIPS I protein shown in [Figure 1]FIGs 1A-1B (last 142 amino acids) (SEQ ID NO:2); and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the HOIPS I protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

The paragraph beginning at page 11, line 3:

In another aspect, the invention provides isolated nucleic acid molecules encoding the HOIPS I polypeptide having an amino acid sequence as encoded by the cDNA clone contained in the plasmid deposited with the ATCC on December 16, 1996 (ATCC Deposit No. 97825). In a further

embodiment, nucleic acid molecules are provided encoding the mature HOIPS I polypeptide or the full-length polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) or the nucleotide sequence of the HOIPS I cDNA contained in the above-described deposited clone, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by *in situ* hybridization with chromosomes, and for detecting expression of the HOIPS I gene in human tissue, for instance, by Northern blot analysis.

The paragraph beginning at page 11, line 16:

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence of the deposited cDNA or the nucleotide sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50, 100, 150, 200, 250, 300, 350, 400, 450, or 500 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1). By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1).

The paragraph beginning at page 13, line 9:

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence of the reference polynucleotide (e.g., the deposited cDNA or the nucleotide sequence as shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1)). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3′ terminal poly(A) tract of the HOIPS I cDNA shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1)), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The paragraph beginning at page 14, line 28:

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to (a) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2; (b) a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO: 2, but lacking the N-terminal methionine; (c) a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions from about 1 to about 142 in [Figure 1]FIGs 1A-1B (SEQ ID NO:2); (d) a nucleotide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97825; (e) a nucleotide sequence encoding the mature HOIPS I polypeptide having the

amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 97825; or (f) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), or (e).

The paragraph beginning at page 20, line 13:

The invention further provides an isolated HOIPS I polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in [Figure 1]FIGs 1A-1B (SEQ ID NO:2), or a peptide or polypeptide comprising a portion of the above polypeptides.

The paragraph beginning at page 37, line 23:

The 5′ oligonucleotide primer has the sequence:

5′ GACTCCATGGGCGGCGGTGGGAAAGCCTG 3′ (SEQ ID NO:4) containing the underlined NcoI restriction site, which encodes 20 nucleotides of the HOIPS I protein coding sequence in [Figure 1]FIGs 1A-1B (SEQ ID NO:1) beginning immediately after the signal peptide.

The paragraph beginning at page 37, line 28:

The 3′ primer has the sequence:

5′ GACTAGATCTGGAGCACATGATAGTAGCAT 3′ (SEQ ID NO:5) containing the underlined BglII restriction site followed by 20 nucleotides complementary to the last 20 nucleotides of the HOIPS I protein coding sequence in [Figure 1]FIGs 1A-1B.

The paragraph beginning at page 40, line 9:

The cDNA sequence encoding the full length HOIPS I protein in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:2), is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GAC TGGATCCGCC ATC ATG AAG GGT TTC ACA GCC AC 3' (SEQ ID NO:6) containing the underlined BamHI restriction enzyme site, an efficient signal for initiation of translation in eukaryotic cells, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), followed by 20 bases of the sequence of the complete HOIPS I protein shown in [Figure 1]FIGs 1A-1B, beginning with the AUG initiation codon. The 3' primer has the sequence 5' GACTGGTACCAG- CAGCTGCACTCTTTGGG 3' (SEQ ID NO: 7) containing the underlined, Asp718 restriction site followed by 19 nucleotides complementary to the 3' noncoding sequence in [Figure 1]FIGs 1A-1B.

The paragraph beginning at page 47, line 13:

The DNA sequence encoding the complete HOIPS I protein including its leader sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' sequences of the gene. The 5' primer has the sequence 5' GACTGGATCCGCCATCATGAAGGGTTTCACAGCCAC 3' (SEQ ID NO:6) containing the underlined BamHI restriction enzyme site followed by an efficient signal for initiation of translation in eukaryotes, as described by Kozak, M., *J. Mol. Biol. 196*:947-950 (1987), and 20 bases of the coding sequence of HOIPS I shown in [Figure 1]FIGs 1A-1B (SEQ ID NO:1). The 3' primer has the sequence 5' GACTGGTACCAGCAGCTGCACTCTTTGGG 3' (SEQ ID NO:10) containing the underlined Asp718 restriction site followed by 19 nucleotides

complementary to the non-translated region of the HOIPS I gene shown in [Figure 1]FIGs 1A-1B

(SEQ ID NO:1).